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Innate immune responses to obesity in cloned and normal outbred domestic pig

Tina Rødgaard[1], Kerstin Skovgaard[1], Jan Stagsted[2], Peter M. H. Heegaard[1]

1: Innate Immunology Group, National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark
2: Department of Food Science – Milk and Egg Science, Aarhus University, Tjele, Denmark

Introduction

Pigs are widely used as a biomedical model for energy metabolism, obesity and inflammation in humans because of the similarities of porcine physiology and metabolic features to humans.
In theory the use of cloned animals should reduce biological variation. Therefore cloning may improve the applicability of pigs as a model for human obesity-induced inflammation.
However, previous findings have suggested that cloning may affect the phenotypes (e.g. [1]) including parts of the innate immune system [2].

Objective To evaluate the effect of cloning on the innate immune response in normal outbred pigs fed a restricted diet (60% of *ad libitum* diet) (lean) (*n*=9) compared to cloned pigs fed an *ad libitum* diet (obese) (*n*=9) or a restricted diet (lean) (*n*=8).

Materials and methods

All experimental procedures involving animals were approved by the Danish Animal Experimental Committee.
Cloning was performed using somatic cell nuclear transfer as described in [3]. Donor cells were from cultured ear fibroblasts obtained from a Danish Landrace x Yorkshire (65%:35%) sow. Normal litters were kept as controls and were obtained after standard artificial insemination.
Pigs were nursed by surrogate sows and weaned after 28 days. For an additional 2 months they were kept on standard diet after which they were individually housed. The pigs were then fed high-energy diet (containing 10% sugar and 10% soy oil) either *ad libitum* or 60% of *ad libitum* consumption. The pigs were killed at 9 months of age and blood and tissue samples were collected.
A phenol/chloroform-method was used to extract mRNA from the tissues. qPCR was performed in 48.48 chips (FluiDigm) on a BioMark real-time PCR instrument (FluiDigm). Data was log2 transformed prior to t-test, F-test and ANOVA.
ELISAs were performed to measure circulating concentrations of selected acute phase proteins.

	Liver			Abdominal SAT			VAT			Neck SAT		
	Control Lean	Clone Lean	Clone Obese	Control Lean	Clone Lean	Clone Obese	Control Lean	Clone Lean	Clone Obese	Control Lean	Clone Lean	Clone Obese
APOA1	1	1,05	0,82	1	1,23	1,34	1	1,38	1,09	1	1,35	1,01
CD14	1	0,96	0,88	1	0,76	0,82	1	0,91	1,09	1	0,80	0,85
CD40	1	1,04	1,03	1	0,76	0,71	1	0,62	0,92	1	1,26	1,15
C3	1	1,02	0,88	1	0,41	0,43	1	0,49	0,30	1	0,95	0,66
CRP	1	1,67	1,27	1	1,06	1,36	1	0,59	0,53	1	0,73	0,90
PDB-2	1	0,92	0,80	1	0,64	0,49	1	0,91	0,99	1	2,62	0,90
FIB	1	1,18	0,93	ODL	ODL	ODL	ODL	ODL	ODL	ODL	ODL	ODL
HP	1	1,28	0,96	1	0,23	0,42	1	0,20	0,13	1	0,92	0,79
IL-8(a)	NQ	NQ	NQ	1	0,58	0,45	1	0,44	0,21	1	3,98	1,47
IL-1B	1	0,89	0,66	1	1,65	0,96	1	0,80	0,83	1	2,07	1,55
IL-6	NQ	NQ	NQ	1	2,04	1,34	1	0,73	0,74	1	1,19	0,43
IL-8(b)	1	0,92	1,46	1	0,57	0,46	1	0,49	0,23	1	3,11	1,62
IL-10(a)	1	1,36	1,21	1	1,13	0,81	1	1,17	1,20	1	2,03	1,26
pigMAP/ITIH4(b)	1	1,61	1,27	1	1,46	1,34	1	1,21	0,86	1	1,25	0,98
pigMAP/ITIH4(a)	1	1,71	1,34	1	1,44	1,61	1	0,87	0,48	1	1,48	1,03
SAA	1	2,59	2,43	1	0,07	0,23	1	0,37	0,38	1	1,27	1,65
TGFB1	1	0,88	0,89	1	0,82	0,87	1	0,75	0,80	1	1,03	1,17
TNF-α	1	1,25	0,90	1	1,58	1,19	1	1,02	1,52	1	1,61	1,33
TLR4	1	0,87	1,04	1	1,15	0,77	1	1,17	1,07	1	1,05	0,85
LBP	1	1,46	1,18	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
AOAH	1	1,17	1,16	1	1,29	1,25	1	1,35	1,52	1	1,66	1,53
CXCL10	1	2,72	1,29	1	1,99	0,71	1	3,04	0,73	1	5,88	0,79
CCL3LI	1	1,11	0,75	1	1,22	0,96	1	0,76	0,64	1	0,96	1,09
CCL2	1	1,06	1,98	1	0,84	0,66	1	0,60	0,51	1	1,10	1,01
CCL5	1	0,99	1,20	1	1,01	1,07	1	1,50	0,77	1	1,40	1,12
PAFA-H1B1	1	0,91	0,92	1	0,87	0,92	1	0,96	1,08	1	0,76	0,91
SFTPA1	1	2,16	3,59	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
IL-1RN	1	0,99	0,86	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
NFKB1	1	0,93	1,07	1	0,83	0,90	1	0,84	0,92	1	0,95	0,92
NFKBIA	1	1,18	0,93	1	1,28	1,05	1	0,97	0,70	1	2,09	1,01
ORM1	1	1,10	1,06	ODL	ODL	ODL	ODL	ODL	ODL	ODL	ODL	ODL
TF	1	1,11	0,99	1	0,49	0,16	1	0,11	0,07	1	1,54	0,86
CD36	1	0,99	0,83	1	1,24	1,49	1	1,26	1,18	1	0,66	0,86
COX-2	1	1,63	4,79	1	1,63	1,02	1	1,33	0,46	1	2,66	0,65
IL-10(b)	1	1,71	1,11	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
CD200	1	0,93	0,93	1	0,72	0,59	1	0,97	0,98	1	0,85	0,79
IL-18	1	0,90	1,33	1	0,67	0,71	1	0,69	1,21	1	2,13	1,93
IFNG	1	0,88	0,96	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
TNFAIP3	1	1,02	0,93	1	1,21	0,97	1	1,14	1,14	1	1,14	0,83
CD163	1	0,94	1,32	1	1,22	1,21	1	1,16	1,35	1	1,53	1,38

0 1 <5

Figure 1: Expression of measured genes in liver, abdominal SAT, VAT and neck SAT in clones and control pigs. Control Lean is set to 1. ODL=Over Detection Limit. NQ=Not Quantifiable.

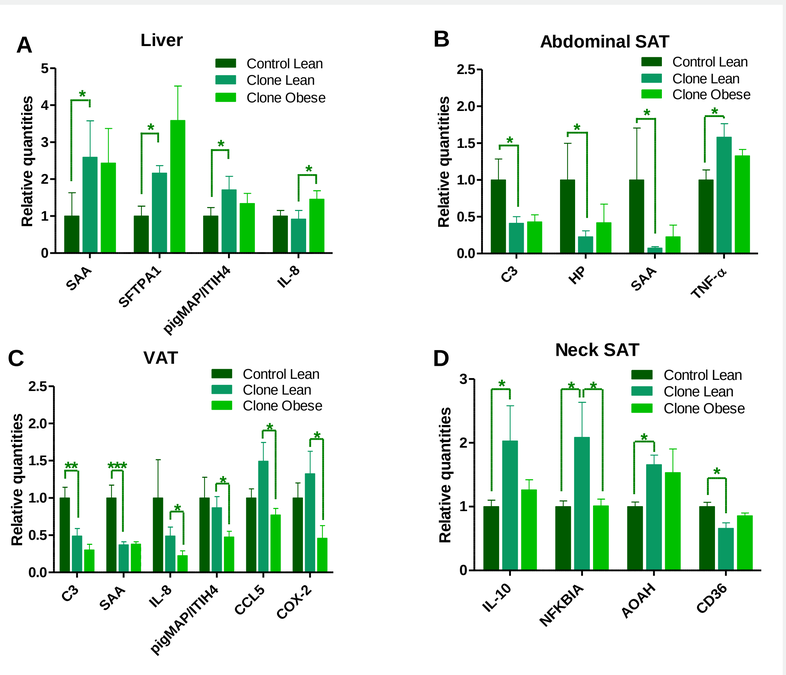

Figure 2: Expression of significant genes in liver (A), subcutaneous fat (SAT) from the abdomen (B), intra-abdominal visceral adipose tissue (VAT) (C) and neck SAT (D) compared to control lean (set to 1) measured with qPCR. Control lean n=9, Clone lean n=8 (except for neck SAT where n=7) and Clone obese n=9. SEM is shown as error bars. *=*P*<0.05, **=*P*<0.01 and ***=*P*<0.001.

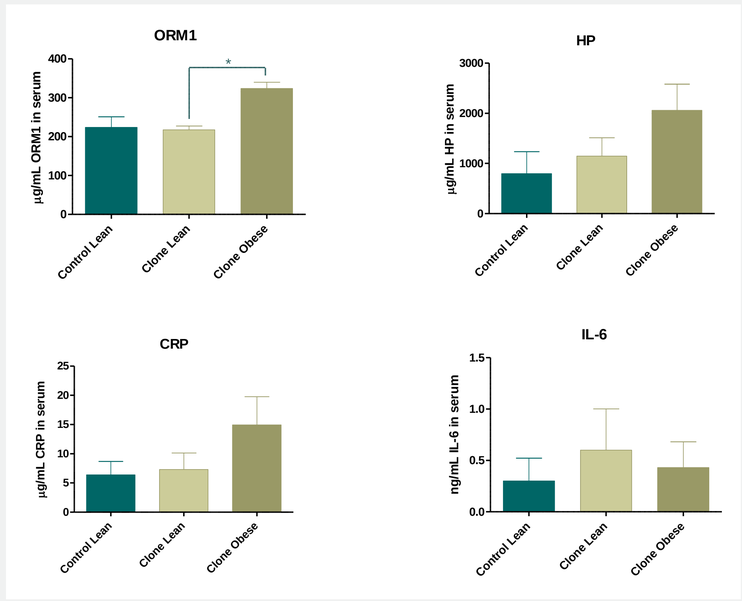

Figure 3: Serum concentrations of ORM1, HP, CRP and IL-6 in controls (n=8, except for ORM 1where n=9) and clones (n=7, except for HP where n=8) measured with ELISAs. SEM is shown as error bars. *=*P*<0.05.

Results and conclusion

The variation in gene expression was found to be similar for the clones and the controls (not shown) and only a limited number of genes were affected by cloning (fig.1 and 2). In the two adipose tissues from the abdominal region (abdominal SAT and VAT(fig.2B and C)) 5 out of 6 significantly differentially expressed genes were down-regulated in the lean clones, whereas most differently expressed genes in both liver (fig.2A) and neck SAT (fig.2D) were up-regulated (6 out of 7). The same pattern was observed in the obese clones, although the number of genes affected was lower.
Systemically, circulating acute phase proteins (HP, CRP and ORM1) had increased serum concentrations in obese but not in lean clones, however this was only statistically significant for ORM1 (fig. 3).
In conclusion, subtle but significant changes were identified in specific subsets of innate immune genes, especially in the lean clones, and this together with the finding that cloned pigs did not show lower inter-individual biological variation reduces the incentive to use cloned pigs for the study of innate immune gene regulation in obesity.

Tina Rødgaard (tinro@vet.dtu.dk)
Innate Immunology Group
National Veterinary Institute
Technical University of Denmark
Bülowsvej 27, Building B, room B7
1870 Frederiksberg C
Denmark

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